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# Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed | |
|---|---|---|
| ☐ | ☒ | The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☒ | ☐ | A description of all covariates tested |
| ☐ | ☒ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ | Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

**Data collection**

Mass cytometry acquisition was performed on DVS Sciences Instrument Control Software 6.0.626.
MIBI acquisition was performed using MiniSIMS 2 software 5.5.4.0

**Data analysis**

The following software and R packages were used for data analysis:

Cytobank.org
Cellengine.com

R version 3.6.0 (2019-04-26)
Platform: x86_64-apple-darwin15.6.0 (64-bit)
Running under: macOS Sierra 10.12.6

effsize_0.7.6
uwot_0.1.4
ggbiplot_0.55
igraph_1.2.4.1
RcppArmadillo_0.9.700.2.0
stringr_1.4.0
tidyverse_1.2.1
RColorBrewer_1.1-2
Matrix_1.2-17
scales_1.1.0
gdata_2.18.0
CytoML_1.10.0
purrr_0.3.2

nationalparkcolors_0.1.0
viridis_0.5.1
magrittr_1.5
plyr_1.8.5
xlsx_0.6.1
pheatmap_1.0.12
readr_1.3.1
ggrastr_0.1.7
viridisLite_0.3.0
scico_1.1.0
reshape2_1.4.3
flowWorkspace_3.32.0
flowCore_1.50.0
tidyr_1.0.0
broom_0.5.2
ggridges_0.5.1
devtools_2.2.0
doBy_4.6-2
ncdfFlow_2.30.1
patchwork_0.0.1
tibble_2.1.3
dplyr_0.8.3
readxl_1.3.1
usethis_1.5.1
FlowSOM_1.16.0
BH_1.72.0-3
forcats_0.4.0
ggplot2_3.2.1
easypackages_0.1.0

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Single-cell mass cytometry (CyTOF) datasets for metabolic analysis of human whole blood populations, in vitro T cell activation and analysis of metabolic states in human tissues as well as MIBI-TOF imaging data of colorectal carcinoma and healthy colon are publicly available at https://doi.org/ 10.5281/zenodo.3951613.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|---|---|
| Sample size | The focus of this study is to describe a novel experimental approach and potential applications for it. Sample sizes were chosen to fulfil these purposes and were not based on mathematical sample size estimations as there was no prior information available to adequately estimate between sample and group differences. For mass cytometry experiments comparing tissue resident metabolic states, the total number of samples was chosen to be 20 to allow for barcoding and combining of all samples into a composite sample. This allowed for joint processing and thus elimination of technical variation between samples. |
| Data exclusions | No samples were excluded. |
| Replication | Replication experiments were performed for several comparisons in this study. Whole blood lineage-specific metabolic profiles were replicated by repeating the experiment with a separate aliquot of cells from the same five donors. Furthermore, we performed a total of 4 independent experiments using different healthy donor cells for experiments demonstrating the correlation between mass cytometry-based protein expression values and Seahorse-based extracellular flux analysis. |
| Randomization | This study did not involve an intervention with control and test groups and therefore, we did not perform randomization. It is an observational study involving comparisons between technologies, samples and within samples (e.g. over time). |

| | |
|---|---|
| Blinding | This study did not involve an intervention with control and test groups and therefore, no blinding was performed. |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology |
| ☒ | ☐ Animals and other organisms |
| ☐ | ☒ Human research participants |
| ☒ | ☐ Clinical data |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

## Antibodies

| | |
|---|---|
| Antibodies used | All antibodies (>110) tested for this study and used in the experiments are described in-depth in Supplementary Table 1 |
| Validation | In addition to supplier-based antibody validations the following workflow was employed:<br><br>To validate metabolic antibodies as broadly as possible, a range of different cell types, tissues and technologies were used in combination. Mass cytometry-based antibody validation was performed on a range of cell lines, immune populations found in whole blood and T cells with or without TCR activation. First, various leukemic, embryonic and carcinoma cell lines were cultured in standard conditions, fixed, palladium-barcoded and subsequently stained with heavy-metal conjugated antibodies as described. Whole blood was processed as before (see above) and stained with a combination of metabolic antibodies and cell lineage markers (CD45, CD3, CD4, CD8, CD45RA, CD66, CD14, CD19, CD20, HLA-DR, CD56, CD57, CD11c, CD123, FceRI, CD235ab) to identify the major immune cell types through manual gating. Human T cells were either rested or activated with anti-CD3/ anti-CD38-beads for 72 h, fixed and palladium-barcoded before staining with metabolic antibodies. Metabolic antibodies were initially used at a concentration of 2 ug/ml. For all populations, median arsinh values were calculated and positive staining was defined as a median of at least 10 ion counts (asinh transformed value >1.5) of any subpopulation. Where available, cell-lineage specific expression and induction upon activation were compared to previously determined values for the given cell population. To validate antibodies on tissues, control tonsil and liver FFPE tissues were stained with the indicated metal-conjugated antibodies as described below and their performance was validated through traditional IHC and MIBI-TOF. Detectable staining was determined through visual inspection of both IHC and grayscale MIBI-TOF images. For intra-assay quality control, IHC and MIBI-TOF images were visually compared and in addition, related to previously determined staining patterns. |

## Eukaryotic cell lines

Policy information about cell lines

| | |
|---|---|
| Cell line source(s) | 293T, A549, HCT-116, HEL, HELA, Jurkat, NCIH460, NTERa, Ramos, REH, THP-1, U937: atcc. ES-H9: WiCell |
| Authentication | No authentication was performed. |
| Mycoplasma contamination | Cell lines were not tested for mycoplasma. |
| Commonly misidentified lines (See ICLAC register) | According to the ICLAC register, some stocks of U937 might be contaminated. However, none of the biological and technical conclusions presented in this manuscript are dependent on data obtained from this cell line. |

## Human research participants

Policy information about studies involving human research participants

| | |
|---|---|
| Population characteristics | All human sample donors are described in Supplementary Table 2. Importantly, this study did not involve any diagnoses specific to age, gender, or race, or draw any conclusions based on these patient characteristics. |
| Recruitment | De-identified peripheral blood samples from healthy human subjects were obtained via the Stanford Blood Center.<br>Colorectal carcinoma and healthy adjacent tissue samples for mass cytometry were obtained post resection from the tissue repository of the Stanford Department of Pathology.<br>FFPE tissue samples of colorectal carcinoma patients and healthy controls were obtained from the tissue repository of the Stanford Department of Pathology.<br>We had no control over the patient samples submitted for diagnostic testing and scheduled for resection and no exclusion criteria were defined. Samples were collected until a sufficient number was reached and subsequently stained and analyzed |

| | |
|---|---|
| | together. |
| Ethics oversight | Samples were obtained and experimental procedures were carried out in accordance with the guidelines of the Stanford Institutional Review Board (IRB). Samples used were obtained as post-diagnostic and post resection excess material prior to disposal, with no change to patient management. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.